

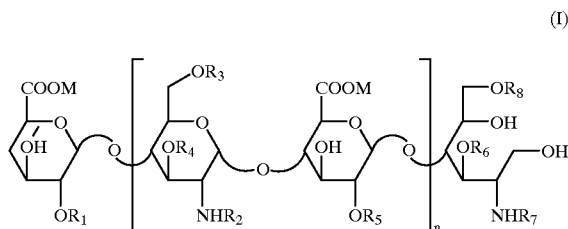
1

**PHARMACEUTICAL COMPOSITIONS  
CONTAINING OLIGOSACCHARIDES, THE  
NOVEL OLIGOSACCHARIDES AND  
PREPARATION THEREOF**

**CROSS REFERENCE TO RELATED  
APPLICATIONS**

This application claims benefit of U.S. Provisional Application No. 60/205,206, filed on May 18, 2000 and of French Patent Application FR0003910 filed on Mar. 28, 2000.

The present invention relates to pharmaceutical compositions containing as active principle an oligosaccharide of formula:



or to a mixture of these oligosaccharides, to the novel oligosaccharides of formula (I), to mixtures thereof and to methods for their preparation.

In formula (I),  $n$  is an integer from 0 to 25,  $R_1$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$  and  $R_8$ , which may be identical or different, represent a hydrogen atom or an  $\text{SO}_3\text{M}$  radical,  $R_2$  and  $R_7$ , which may be identical or different, represent a hydrogen atom or an  $\text{SO}_3\text{M}$  or  $\text{COCH}_3$  radical, and  $M$  is sodium, calcium, magnesium or potassium.

These oligosaccharides thus comprise an even number of saccharides.

In formula (I),  $R_4$  and  $R_6$  are, preferably, hydrogen atoms.

Oligosaccharides of formula (I) for which  $n$  is equal to 0, and either  $R_1$ ,  $R_6$  and  $R_8$  represent a hydrogen atom,  $R_7$  represents an  $\text{SO}_3\text{M}$  or  $\text{COCH}_3$  radical and  $M$  is sodium, or  $R_1$  and  $R_6$  represent a hydrogen atom,  $R_7$  represents a  $\text{COCH}_3$  radical,  $R_8$  represents an  $\text{SO}_3\text{M}$  radical and  $M$  is sodium, or  $R_6$  represents a hydrogen atom,  $R_1$ ,  $R_7$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical and  $M$  is sodium have already been described by G. H. LEE et al., J. Chromat. 212, 65-73 (1981), but no pharmacological property is described for these products.

Oligosaccharides of formula (I) for which  $n$  is equal to 0, and either  $R_6$  and  $R_7$  represent hydrogen atoms,  $R_1$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical and  $M$  is sodium, or  $R_1$ ,  $R_6$  and  $R_7$  represent a hydrogen atom,  $R_8$  represents an  $\text{SO}_3\text{M}$  radical and  $M$  is sodium, are described by M W McLEAN et al., Eur. J. Biochem., 1984, 145, 607, without any indication of pharmacological activity.

The pharmaceutical compositions which are preferred are those containing an oligosaccharide of formula (I) for which:

$n$  is an integer from 0 to 10, and in particular from 0 to 6, and even more particularly from 1 to 6.

$R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_7$  and  $R_8$  are identical or different, and represent a hydrogen atom or an  $\text{SO}_3\text{M}$  radical, and in particular  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_7$  and  $R_8$  are  $\text{SO}_3\text{M}$  radicals,  $M$  is sodium.

The pharmaceutical compositions which are particularly preferred are those containing an oligosaccharide of formula (I) for which:

$n$  is equal to 0,  $R_1$ ,  $R_7$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical,  $R_6$  represents a hydrogen atom and  $M$  is sodium,

2

$n$  is equal to 1,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_7$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical,  $R_4$  and  $R_6$  represent a hydrogen atom and  $M$  is sodium,

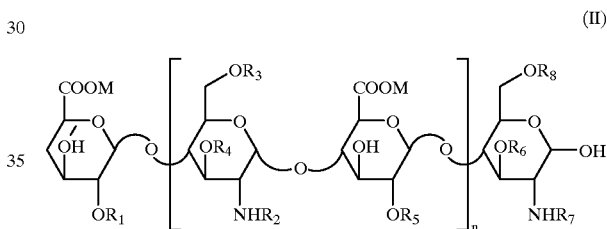
$n$  is equal to 2,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_7$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical,  $R_4$  and  $R_6$  represent a hydrogen atom and  $M$  is sodium,

$n$  is equal to 3,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_7$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical,  $R_4$  and  $R_6$  represent a hydrogen atom and  $M$  is sodium,

$n$  is equal to 4,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_7$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical,  $R_4$  and  $R_6$  represent a hydrogen atom and  $M$  is sodium.

The oligosaccharides of formula (I), with the exception of those for which  $n$  is equal to 0 and either  $R_1$ ,  $R_6$  and  $R_8$  represent a hydrogen atom,  $R_7$  represents an  $\text{SO}_3\text{M}$  or  $\text{COCH}_3$  radical and  $M$  is sodium, or  $R_1$  and  $R_6$  represent a hydrogen atom,  $R_7$  represents a  $\text{COCH}_3$  radical,  $R_8$  represents an  $\text{SO}_3\text{M}$  radical and  $M$  is sodium, or  $R_6$  represents a hydrogen atom,  $R_1$ ,  $R_7$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical and  $M$  is sodium, or  $R_6$  and  $R_7$  represent hydrogen atoms,  $R_1$  and  $R_8$  represent an  $\text{SO}_3\text{M}$  radical and  $M$  is sodium, or  $R_1$ ,  $R_6$  and  $R_7$  represent a hydrogen atom,  $R_8$  represents an  $\text{SO}_3\text{M}$  radical and  $M$  is sodium, are novel and, as such, form part of the invention.

The oligosaccharides of formula (I) can be prepared by reaction of an alkali metal borohydride or a quaternary ammonium borohydride with oligosaccharides of formula:



in which  $n$  is an integer from 0 to 25,  $R_1$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$  and  $R_8$ , which may be identical or different, represent a hydrogen atom or an  $\text{SO}_3\text{M}$  radical,  $R_2$  and  $R_7$ , which may be identical or different, represent a hydrogen atom or an  $\text{SO}_3\text{M}$  or  $\text{COCH}_3$  radical, and  $M$  is sodium, calcium, magnesium or potassium.

This reaction is carried out in aqueous medium, at a temperature in the vicinity of  $25^\circ\text{C}$ ., at a pH between 7 and 10, and preferably between 9 and 10, for the entire duration of the reaction. The pH is maintained by addition of a sodium hydroxide solution at 0.5 mol/l. The reaction is stopped by acidification of the reaction medium, for example by addition of acetic acid until a pH between 4 and 5 is obtained.

As alkali metal borohydrides, mention may be made of lithium borohydride, sodium borohydride and potassium borohydride.

As a quaternary ammonium borohydride, mention may be made of tetrabutylammonium borohydride.

The oligosaccharides of formula (II) can be obtained by gel chromatography separation of a mixture of oligosaccharides (III) obtained by enzymatic depolymerization of heparin or basic depolymerization of the benzyl ester of heparin or of a benzyl ester of semi-synthetic heparin.

This chromatography is carried out on columns filled with gel of polyacrylamide-agarose type, such as that sold under the trade mark Ultrogel ACA202<sup>R</sup> (Biosepra). Preferably, an array of polyacrylamide agarose gel columns is used. The